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10/715,231	11/17/2003	Tariq M. Rana	UMY-049	5030

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EXAMINER	
WOOLWINE, SAMUEL C	

ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 01/03/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/715,231	RANA, TARIQ M.	
	Examiner	Art Unit	
	Samuel Woolwine	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-28 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-28 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Specification

The disclosure is objected to because of the following informalities: On page 9, lines 3-5 and page 37, lines 13-19, the specification refers to "standard methods" and "routine methods", respectively, for extending the 5' end of the miRNA toward the 3' end of the target RNA to which said miRNA is hybridized, citing Wang et al., *Biochemistry*, 40:6458-6464 (2001). The Wang reference does not discuss this technique, and the Examiner is unaware of any means known in the art by which such a template-directed "5' fill-in" reaction can be accomplished.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-28 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for practicing the invention with a defined miRNA and a suspected or known target, does not reasonably provide enablement for identifying miRNAs and their targets when neither is known *a priori*. Nor does the specification reasonably provide enablement for practicing the invention with complex mixtures of RNA species. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention

The claims are drawn to methods of identifying miRNAs and their targets, as well as the use of miRNAs so identified to alter the expression of its target gene in living cells. The invention is an class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." Mycogen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Fed. Cir. 2001).

The breadth of the claims

The claims broadly encompass the identification of any miRNA and its target(s) in any species which forms miRNAs including plants, animals, fungi and other single-celled organisms. The methods of claims 1-4 in particular encompass the situation in which neither the miRNA nor the target RNA(s) are known in advance. The claims encompass the practice of the invention with complex mixtures of undefined RNA species.

Quantity of Experimenation

The quantity of experimentation in this area is large since the data generated from practicing the invention with complex mixtures of undefined RNA species would be expected to contain, in addition to the desired sequence information corresponding to miRNAs and their targets, a significant amount of data that does not correspond to miRNAs. To accomplish the goal of the method, i.e. the identification of miRNAs and their targets, one would have to determine which data corresponds to miRNA and which does not. All pending claims rely on the ability of the miRNA to hybridize at its 3' end to a target RNA to allow for the enzyme-catalyzed extension of said miRNA in a 5' to 3' manner. However, *any* appropriately base-paired 3' terminus would result in extension, including RNA molecules which fold back on themselves in a "hairpin" or similar secondary structure, as well as any RNA (including miRNA) that has sufficient complementarity at its 3' end to another RNA molecule to allow for hybridization. Such non-specific hybridization would be expected to generate a large amount of spurious data that would require a great deal of further experimentation to identify small RNAs that actually represent miRNA. Additionally, in the case where no prior knowledge of the sequence of the miRNA or the target exists, Applicant's methods result in the identification of a sequence that is a chimera of the miRNA and its target. Unless the entire genome sequence of the organism from which the RNA was derived is available, a great deal of further experimentation would be needed to parse the sequence data in order to identify what was the miRNA and what was the target.

The unpredictability of the art and the state of the prior art

Some of the unpredictability of the art has been touched on in the preceding paragraph. Specifically, one could not predict, in the case of complex mixtures of RNA, which products resulting from Applicant's methods correspond to true miRNA/targets and which do not. Applicant describes the purification of miRNA from total RNA on page 35 of the specification, lines 8-10: "The presence of miRNA is confirmed, e.g., by separation on a denaturing 15% polyacrylamide gel, and the miRNA bands are excised and purified by routine methods." Fu et al (2005) attempted to clone miRNA from human fetal liver total RNA by size fractionation (see page 3850, *Materials and Methods* paragraphs 2.2 and 2.3). Fu found that "[m]ore than 60% of the cloned RNAs represented breakdown products of abundant non-coding RNAs such as tRNA, rRNA, snRNA, and snoRNA. Some of them represented known miRNAs." Therefore, even miRNA "purified" from total RNA according to Applicant's instructions would contain a large amount of contaminating small RNAs which, together with a complex and undefined mixture of RNA comprising bona fide "target" RNA, would generate unpredictable results which would require a great deal of further experimentation to allow for the identification of true miRNA and target RNA. Compounding this problem is the fact that in many cases, the 3' end of the miRNA is not complementary to its target (see Lai, 2002, figure 1d, only 1 out of 4 cases shows the 3'-most nucleotide to the miRNA to be complementary to the target). To reiterate, all of the pending claims recite

methods that rely on the ability of the miRNA to hybridize at its 3' end to a target RNA to allow for the enzyme-catalyzed extension of said miRNA in a 5' to 3' manner.

Applicant admits to the uncertain state of the art on page 1, lines 22-25: "Many miRNAs appear to be evolutionarily conserved across species from worms to humans, and are *believed to act by hybridizing* with a target RNA. By a mechanism that *is not completely understood*, this interaction results in post-translational suppression of the target genes." (emphasis added). The unknown nature of the mechanism by which miRNAs act is particularly relevant to claims 26-28 in which Applicant proposes to regulate gene expression using miRNAs. An additional issue of unpredictability of the use of miRNAs to regulate gene expression as recited in claims 26-28 is raised by Tomari (2005):

> "miRNAs are cousins of siRNAs: They are endogenous small RNA guides that repress the expression of target genes. MiRNAs differ from siRNAs in their biogenesis, not in their functions. Like siRNAs, plant and animal miRNAs can direct cleavage of their mRNA targets when the two are extensively complementary, but repress mRNA translation when they are not." (page 518, column 2, 36-53, citations omitted)

> "In fact, complete pairing of the 3' half of an miRNA or siRNA to its target RNA is not required for translational repression, provided that multiple small RNAs are bound to the target, nor for target mRNA destruction, if the bases surrounding the scissile phosphate can form an A-form helix locally. Technologically, this means that most active siRNAs will not only down-regulate their intended mRNA targets but also reduce expression of other mRNAs possessing partial complementarity to the siRNA guide strand" (page 522, column 2, last 4 lines through page 523, column 1, first 7 lines, citations omitted)

Working Examples

The specification provides "theoretical" examples of how the method should work, but there are no actual working examples.

Guidance in the Specification.

The specification provides general guidance on carrying out the methods. However, this guidance does not overcome or even address the technical problems such as contaminating small RNAs in miRNA-containing fractions derived from total RNA, how to practice the methods to identify the majority of miRNAs which do not exhibit target complementarity at the 3' end of said miRNA, how to discriminate between true miRNA/target sequences and sequences generated by non-specific priming from any hybridized 3' end of an RNA molecule, how to, in the case of a sequence which does represent a true miRNA/target interaction, discern what portion of the sequence represents the miRNA and what portion represents the target in the absence of any prior knowledge of either the target or the miRNA, or how to prevent the alteration in the expression of non-target genes by an miRNA, as raised by Tomari (supra).

Additionally, the guidance in the specification makes reference to a method for extending the 5' end of the miRNA toward the 3' end of the target RNA to which said miRNA is hybridized, citing Wang et al., *Biochemistry*, 40:6458-6464 (2001). The Wang reference does not discuss this technique, and the Examiner is unaware of any means known in the art by which such a template-directed "5' fill-in" reaction can be accomplished. The specification also teaches the ligation of linkers or adapters onto the ends of the miRNA/target RNA complex. While ligation of adapters to blunt-ended double-stranded nucleic acid is known in the art, the ligation of linkers or adapters to single-stranded ends of double-stranded nucleic acid (see, e.g., figures 1A, 1B, 2A, 2B,

3 and 4 of the instant application) in cases where there is no knowledge of the sequence of the nucleic acid molecules is a technical challenge not adequately dealt with in the specification.

Level of Skill in the Art

The level of skill in the art is deemed to be high.

Conclusion

The claims of the instant application are broadly drawn to identifying any miRNA/target pair. All pending claims rely on the ability of the miRNA to hybridize at its 3' end to a target RNA to allow for the enzyme-catalyzed extension of said miRNA in a 5' to 3' manner. The art teaches that many, if not most, miRNAs could not be identified by the claimed methods because the do not exhibit complementarity to their target at the 3' end, which would be required in order for the enzyme-catalyzed extension of said miRNA in a 5' to 3' manner. The claims broadly encompass the use of complex; undefined mixtures of RNA comprising miRNA, targets, and non-specific RNA, but the specification does not teach one of skill in the art how to identify the minority of true miRNA/target sequences from non-specific products that would be generated by practicing the claimed methods. Finally, for those products produced by the claimed methods which do represent true miRNA/target complexes, the specification does not enable one of skill in the art to discern what portion of the sequence represents the miRNA and what portion represents the target in the absence of any prior knowledge of

either the target or the miRNA. Even thought the level of skill in the art is high, one of skill in the art would need to carry out a significant amount of undue experimentation to use the invention commensurate in scope with these claims.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1 and 9 are rejected under 35 U.S.C. 102(b) as being anticipated by Wells et al (2001). With regard to claim 1, Wells teaches a method comprising: *(a) obtaining an miRNA/target RNA complex* (page 123, section 2.9, lines 1-4), *(b) optionally crosslinking the complex* (since this limitation is optional, the method taught by Wells applies), *(c) transcribing target complementary RNA (tcRNA) from the target RNA* (see page 123, section 2.9, lines 5-18), *(d) synthesizing cDNA complementary to the tcRNA* (see page 123, sections 2.10 and 2.11), *(e) sequencing the cDNA* (see page 124, section 2.15).

With regard to claim 9, all the steps of the method taught by Wells are carried out in a cell-free system.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Samuel Woolwine whose telephone number is (571) 272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

scw

JEFFREY FREDMAN
PRIMARY EXAMINER
12/11/12